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Short Communication

Sites of Gibberellin Biosynthesis in Pea Seedlings[1]

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ABSTRACT

Potential sites of gibberellin biosynthesis in 10-day-old 'Alaska' pea (*Pisum sativum* L.) seedlings were investigated using a cell-free ezyme system capable of incorporating [^{14}C]-mevalonic acid into *ent*-kaurene. In peas, *ent*-kaurene is assumed to be a committed intermediate in the gibberellin biosynthetic pathway. Comparative results from enzyme assays using extracts from shoot tips, leaf blades, internodes, and root tips indicate that the highest capacity for *ent*-kaurene (and presumably gibberellin) synthesis is in those tissues with the greatest potential for growth. The highest rates were obtained with extracts prepared from the fifth (youngest) internode, the fourth (youngest) expanded leaf, and the shoot tip itself. This report represents the first direct evidence that the enzymes responsible for early stages in gibberellin biosynthesis occur in internode tissues with potential for rapid elongation.

The gibberellins (GAs) elicit a large number of physiological responses in higher plants. The best known activities of GAs is their ability to stimulate internode elongation. A full understanding of the role(s) GAs play in growth regulation will require more comprehensive knowledge of their mechanism(s) of action, relative rates of biosynthesis, transport and metabolism, and their sites of biosynthesis in the growing plant.

Results from a number of previous investigations using a variety of techniques have all led to the conclusion that the shoot tip is a primary site of GA biosynthesis in vegetative parts of peas and other dicotyledonous plants. The initial excision experiments of Lockhart (23), the combined extraction-diffusion experiments conducted to Jones and Lang (18), and the experiments of Moore and Ecklund (7, 24) using applications of growth regulators are all consistent with this hypothesis. Likewise, results of diffusion and extraction experiments suggest the most active site of synthesis in sunflower plants, *Helianthus annuus*, is confined to the young expanding petiolate leaves of the apical bud, rather than to the apical dome and leaf primordia (17), and the developing primary leaves of cowpea seedlings, *Vigna sinensis*, appear to be a major source of GAs (11). In all of these plants and in garden beans, *Phaseolus vulgaris* (22), the growth rate of the internode immediately below these young leaves is strongly correlated with the unfolding and development of the leaves, and presumably dependent upon the flow of GAs from their site of synthesis into the elongating internode.

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All of these results, however, are based upon relatively indirect measurements of GAs extracted or diffused from tissues or the growth response of plants to applied GAs or growth regulators. The use of a cell-free enzyme system from pea seedling shoot tips which is capable of catalyzing certain key reactions in the GA biosynthetic pathway (Fig. 1), represents an independent and somewhat more direct avenue for examination of potential sites of GA biosynthesis. Specifically, the cell-free incorporation of [^{14}C]-MVA[2] into *ent*-kaurene (an intermediate which appears to be committed to GA biosynthesis) can be considered a reasonably good indication of the potential for GA biosynthesis in tissues from which the extracts are made. Thus, previous reports on such systems from developing seeds, fruits, and seedling shoot tips (1, 5, 13) have provided evidence that these are sites of GA biosynthesis. The present paper describes experiments designed to compare the relative rates of incorporation of [^{14}C]-MVA into *ent*-kaurene in cell-free extracts from several seedling parts and thereby to determine if vegetative tissues other than the shoot tip also have the potential to produce GAs.

MATERIALS AND METHODS

High speed supernatant fractions from 10-d-old 'Alaska' pea shoot tips have previously been shown to incorporate [^{14}C]-MVA into *ent*-kaurene when incubated in reaction mixtures containing 20 μM [2-^{14}C]-MVA, 3 mM ATP, 2 mM Mg^{2+}, and 2 mM Mn^{2+} at pH 7.1 (5). Using essentially the same methods (except that 150,000*g* supernatants were dialyzed for 2 h in most cases), extracts were prepared for this study from excised leaf blades, internodes, apical buds, and root tips of 10-d-old Alaska pea seedlings as indicated in Figure 2. Reactions containing 400 μl enzyme extract (linear through 600 μl) were allowed to proceed for 60 min (linear through 90 min) at 30°C and the [^{14}C]-*ent*-kaurene formed was determined by liquid scintillation spectrometry after separation by TLC (5). R-[2-^{14}C]-MVA (23 μCi/μmol) was obtained from Amersham; ATP was from Sigma. Protein determinations were based on the method of Bradford (3) using the Bio-Rad protein assay kit.

RESULTS AND DISCUSSION

The comparative accumulations of [^{14}C]-*ent*-kaurene in extracts from different parts of 10-d-old pea seedlings are listed in Table I. These data, which are representative of those from three similar experiments, demonstrate that enzymes capable of incorporating MVA into *ent*-kaurene are present in all aerial parts tested. The fourth and last unfolded leaf and the apical bud from 10-d-old plants has great potential for *ent*-kaurene biosynthesis. The fifth internode, which lies between the fourth leaf and the apical bud, also exhibits a high level of activity. The activity declines in lower internodes and leaves and is virtually undetect-

[2] Abbreviation: MVA, mevalonic acid.

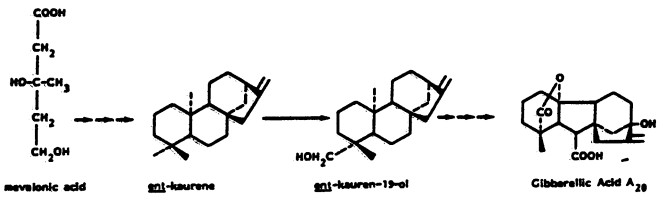

FIG. 1. Pathway of gibberellin biosynthesis from MVA. GA_{20} is illustrated as it is one of the endogenous GAs in peas.

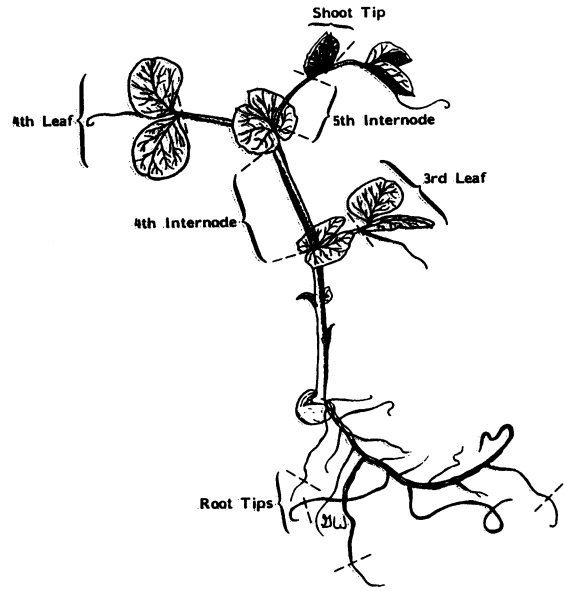

FIG. 2. Drawing of 10-d-old Alaska pea seedling. Plant parts excised, extracted, and assayed for enzymes are indicated by brackets.

Table I. *ent-Kaurene Biosynthesis in Extracts of Pea Tissues*

Dialyzed 150,000*g* supernatants were prepared from each of the six plant parts listed (Fig. 2) after excision from 10-d-old Alaska pea seedlings. Tissues were homogenzied in 50 mM TES buffer containing 5 mM potassium phosphate (1.0 ml per g fresh weight tissue). Reaction mixtures contained 400 μl enzyme extract, 20 nmol R-[2-^{14}C]-MVA (23 μCi/μmol), 3 μmol ATP, 2 μmol Mn^{2+}, 2 μmol Mg^{2+}, and 425 μl buffer in a total volume of 1.0 ml at pH 7.1. Reactions were incubated 60 min at 30°C and [^{14}C]-*ent*-kaurene formed was determined by liquid scintillation spectrometry. Quantities presented are mean values from duplicate reaction mixtures with background from zero-time controls subtracted.

Tissue	*ent*-Kaurene	Protein	*ent*-Kaurene Protein
	pmol	*mg*	*pmol mg*
Shoot tip	7.02	3.48	2.02
5th internode	18.26	2.08	8.78
4th leaf	15.12	4.56	3.32
4th internode	1.63	1.12	145
3rd leaf	1.38	1.64	0.84
Root tip	0.12	0.79	0.16

able in root apices. It should be noted that the activities reported in Table I are all approximately twice those obtained with undialyzed samples of the same enzyme extracts (data not shown). This indicates the presence of a dialyzable inhibitor in these extracts which may be similar to that reported by Shen-Miller and West (28) in extracts of sunflower plants or that reported by Gafni and Shechter (9, 10). Further efforts to exclude the inhibitor or determine its nature were not made.

In experiments reported here with cell-free enzyme systems, the shoot tip, a continually changing entity, consisted of tissue less than 1 cm in length which represented the youngest leaf pair as well as the apical meristem enclosed in stipules (see Fig. 2). However, the blades of the unfolding fifth leaf were not tested for *ent*-kaurene synthesizing activity. Ecklund and Moore (8) previously demonstrated that the capacity for *ent*-kaurene biosynthesis in cell-free extracts from pea shoot tips reaches a maximum when the seedlings are approximately 9 d old and then remains relatively constant through the 24th d. It is, therefore, not surprising that the shoot tip and even the fourth and youngest unfolded leaf in 10-d-old peas contain enzymes capable of incorporating [^{14}C]-MVA into *ent*-kaurene.

The measurement of relatively high levels of enzyme activity in extracts from the internode sections was unexpected. The plants used in these experiments were harvested at a time when the 5th internode was approximately 10 mm in length. Measurements made on intact plants showed that the 5th internode elongated to approximately 20 mm by the 11th d and 30 mm by the 12th d. The 4th internode, on the other hand, had already exhausted much of its growth potential by the 10th d.

Jones and Phillips (17) reported diffusable GA-like activity was obtained from internode sections of sunflower plants. But the level was substantially lower than in young leaves, and it was not at all clear that the internodes were contributing to GA synthesis. Kaufman *et al.* (21) analyzed the GAs in extracts of various parts of oat plants, *Avena*, and reported relatively small amounts in young internodes as compared to the inflorescences and nodes. They were also able to show some interesting qualitative changes in the GAs in internodes at different stages of growth. Although the nonpolar GA-like substances (presumably precursors to more polar biologically active GAs) remained relatively constant during the lag, log, and plateau stages of internode growth, GA_3 content was highest in log and plateau stages. These results indicate that later steps in the GA biosynthetic pathway may be operating in oat internode tissues. It also seems likely that *ent*-kaurene synthesized in pea internodes is further converted to GAs in this tissue since *ent*-kaurene is very nonpolar and is synthesized in the most actively growing tissues.

Jones and Phillips (17) presented evidence that root tips are a site of GA synthesis in sunflowers. Their result was consistent with the report by Sitton *et al.* (29) that [^{14}C]-MVA was incorporated into a product which behaved chromatographically like *ent*-kaurenol (the hydroxylation product of *ent*-kaurene, see Fig. 1) in excised sunflower root tips. In the present study, extracts of 1-cm sections of pea root tips did not catalyze the incorporation of significant amounts of [^{14}C]-MVA into *ent*-kaurene under conditions where extracts from other organs did so (Table I). Although the optimum conditions of the cell-free system (ATP, Mg^{2+}, Mn^{2+}, MVA, and enzyme concentrations) from shoot tips, leaves and internodes are virtually identical (data not shown), it is possible that these are not the optimum conditions for root tip preparations or that *ent*-kaurene biosynthesis is suppressed in root-tip extracts by inhibitors. Thus, the present results do not exclude pea root tips as a possible site of GA synthesis.

Similar cell-free enzyme extracts from pea seeds and fruits (1, 4, 13) have been described previously and indicate that GA biosynthesis is occurring in these reproductive tissues. Indeed, preparations from immature pea seeds yield rates of *ent*-kaurene synthesis about 50 times greater than those from shoot tips (5) and have been shown to catalyze the reactions leading from *ent*-kaurene to the major pea gibberellins (19).

The results presented here provide evidence that enzymes responsible for the incorporation of [^{14}C]-MVA into *ent*-kaurene are present in extracts of excised pea shoot tips, leaves, and internodes. Based upon the comparative rates of *ent*-kaurene accumulation, it appears that the rate of GA synthesis is strongly correlated with the growth potential of the tissue, *i.e.* in the shoot tip as its young leaves are expanding, in the young developing

leaves, and in the elongating internode. *ent*-Kaurene synthesis is greatly diminished in extracts of older leaves and internodes where growth has declined or stopped.

The correlations between growth and cell-free *ent*-kaurene synthesis in peas have been cited previously (4, 5, 8). In the present case, similar correlations can be drawn as they relate to internode elongation and leaf expansion. However, these data do not preclude a contribution of GAs synthesized in adjacent leaves having an effect on the growth of internodes. Indeed, such effects are suggested by results of other types of experiments (7, 11, 17, 22, 23). But, the finding that enzymes capable of *ent*-kaurene synthesis are present in relatively high quantities in elongating internodes does raise questions about the relative contributions to growth of GAs which may be synthesized within a tissue, relative to those transported to the tissue. Recently, some of the native GAs of vegetative tissues of peas have been identified (6, 12) and much progress has been made by characterizing the gibberellins, their metabolism, and their transport in a series of stem-length mutants of peas (12, 16, 25–27). In addition, Ingram *et al.* (15) have now provided evidence from feeding studies that the Le locus in peas which determines either tall (Le-) or dwarf (le/le) controls the 3β-hydroxylation of GA_{20} to GA_1. It will be of great interest to see if the distribution of the 3β-hydroxylase and other enzymes in this pathway beyond *ent*-kaurene in pea seedlings is similar to the distribution of *ent*-kaurene-synthesizing enzymes as described here.

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